

Nutritional value of microalgae for ruminants and implications from microalgae production

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Abstract

Microalgae are a diverse group of microorganisms that are an interesting alternative feed resource for ruminant production. Microalgae species with high protein concentration and adequate amino acid (AA) composition can be used to substitute conventional protein feeds, whereas species with high carbohydrate or lipid concentration can be used to supply energy. Microalgal polyunsaturated acids and short-chain fatty acids have potential to improve the nutritive value of ruminant milk and meat for human consumption and mitigate enteric methane emissions. Microalgae composition is very plastid in comparison to conventional ruminant feeds and it can be influenced relatively easily by environmental conditions, such as nutrient supply. Microalgae also contain many compounds, especially carbohydrates and cell coverings, which are not usually found in ruminant feeds. Standard feed evaluation methods involving the use of crucibles or nylon bags (detergent fibre method, *in vitro* digestibility and *in vivo* rumen incubation) suit poorly to the analysis of microalgae with microscopic particle size. This paper attempts to give a general overview of the nutritive value (protein, lipids and carbohydrates) of microalgae for ruminant feeding applications and the possibilities to tailor microalgae composition for a certain ruminant feeding objectives. In addition, the key knowledge gaps related to the nutritive value of microalgae for ruminant nutrition are identified.

Keywords: microalgae, ruminants, nutrition, nutritive value, microalgae production

Review Methodology: The following databases were used for the literature research: Scopus (Elsevier, Amsterdam, the Netherlands), Web of Science (Thomson Reuters Science, New York, USA) and Google Scholar (Google LLC, Mountain View, USA). Main keywords used in search are: cow, ruminant, microalgae and specific names of different microalgae species. In addition, backward reference searching (i.e. identifying the references cited in the articles obtained by the method described above) and forward reference searching (i.e. identifying articles that cite the articles obtained by the method described above) were used.

Introduction

Microalgae, here understood as both prokaryotic and eukaryotic, mostly photosynthetic unicellular organisms (also known as phytoplankton), are a very interesting alternative feed resource for ruminant production. Microalgae have rapid growth rate [1, 2] and high concentration of interesting nutritive components (e.g. protein, unsaturated fatty acids (FA) and antioxidants) [3].

The protein yields of microalgae have exceeded those of conventional protein feeds by factor of 2–25 in North-Western Europe [4]. Moreover, in microalgae production, it is possible to utilise and recycle production inputs (e.g. nutrients, flue gases, waste and salt water) outside of conventional food and feed production chains [5]. Microalgae can also be produced on marginal or non-arable land, which reduces the competition with edible crops [1]. Microalgae are a highly diverse group of

organisms found in different biological kingdoms (Eubacteria, Plantae, Chromistae, Protists and Fungi) [6] and adapted to various environmental conditions [7, 8]. This is also reflected to the nutritive value of microalgae, which is highly variable between and within species (Table 1). The difficulties with defining what an 'alga' is (e.g. some references only accept photosynthetic eukaryotic species as algae), and complex and constantly changing taxonomy further complicate the picture [89, 90]. For example, fungi-like heterotrophic microorganism *Aurantiochytrium limacinum* may or may not be considered as an microalga, depending on the source of information. The complexity of microalgal components such as cell wall, storage polysaccharides and various organelles also reflects the evolutionary history of these species. The evolutionary oldest microalgae, cyanobacteria, are typically relatively simple in structure, and the complexity has increased with evolution in primary endosymbiosis (resulting in e.g. Chlorophytes and Rhodophytes) and even more in secondary endosymbiosis (resulting in e.g. Euglenophytes and Haptophytes) [91].

Microalgae are cultivated in open (raceway ponds) or closed systems (photobioreactors and fermenters) under autotrophic (with the presence of CO₂ and sunlight) or heterotrophic conditions (with the presence of organic carbon and absence of sunlight) [92]. The chemical composition of microalgae can be manipulated relatively easily by altering the growth medium and growing conditions (e.g. temperature, pH, salinity and availability of light and different nutrients) [93–96]. This is a great advantage, because on terrestrial plants, usually heavier, more laborious and expensive methods, such as breeding, genetic manipulation and processing, are needed in order to trigger major changes in the chemical composition of biomass. This diversity and possibility to influence the composition (i.e. plasticity) of microalgae enables multiple different utilisation possibilities in animal nutrition, but also tailoring microalgae to fit optimally to certain livestock production targets. Locally produced, productive and sustainable protein feeds and feeds with ability to decrease enteric methane emissions are urgently needed in ruminant nutrition. The current mainstream protein feeds (e.g. soybean and rapeseed meals) are well fitted to animal nutrition, but their production and consumption are burdened with various sustainability challenges [97, 98]. Moreover, the low protein self-sufficiency of European Union [99] makes livestock sector vulnerable to trade distortions, availability and price volatility of imported protein feeds [100, 101].

The utilisation of microalgae rich in unsaturated fat as ruminant feed can also generate direct health benefits for humans by decreasing the concentration of saturated fatty acids (SFA) and increasing that of polyunsaturated fatty acids (PUFA) in milk and meat [27, 102]. Diets high in lipids, especially 12:0, 14:0, 18:1 n-9, 18:2 n-6 and 18:3 n-3, have also potential to decrease enteric methane production of ruminants [103]. Microalgae rich in lipids or carbohydrates

can be utilised as energy feeds, and species with high concentration of antioxidants or minerals (e.g. iodine, selenium) to supplement dairy cow diets aiming to improve for example animal health or nutritive quality of milk or meat.

The high production cost of microalgae currently hinders their large-scale utilisation in animal nutrition. The estimated biomass cost of microalgae (e.g. 0.42–5.1 €/kg as summarised by [104]) still largely exceeds the world market price of soya bean meal (on average 0.34 €/kg during 2014–2020; [99]). However, microalgae production price compares more favourably to the market price of fishmeal and oil (1–1.9 €/kg in European Union during 2012–2018; [105]); therefore microalgae likely first establishes itself in animal nutrition as a substitute of aquatic protein and lipids. With further technical development and the use of waste resources in microalgae production, it is possible to decrease the production cost of microalgae [106].

The lipid-extracted microalgae biomass derived from production of, for example, biofuels or dietary supplements are high-value co- or by-products for animal nutrition, which can also improve the economic feasibility of microalgae biofuels [107]. Moreover, total mixed ration feeding used in ruminant nutrition allows the utilisation of feed resources with relatively low dry matter (DM) concentration, such as fresh microalgae paste. Also delivering microalgae to ruminants via drinking water is feasible [108]. Thus, the drying and dewatering of microalgae biomass, which contributes significantly to the energy consumption of microalgae production [109], is not always necessary when utilised in ruminant production. However, the hygienic quality of fresh microalgae paste should be ensured with quick consumption after harvesting (i.e. close proximity of microalgae production and consumption sites), or the use of preservatives. In this review, the research on the nutritive value of microalgae is summarised on the perspective of ruminant nutrition, and the impacts of microalgae production practices on these characteristics are discussed. The composition of industrially most relevant microalgae genus [6] and species commonly studied in animal nutrition are reviewed with emphasis on macronutrients (protein, lipids and carbohydrates). Although microalgae contain also other compounds (e.g. pigments, antioxidants and minerals) potential to generate value-added animal products, they are out of scope of this review.

Protein quantity and quality

The nitrogen (N) concentration of microalgae has varied from 5 to 114 g/kg DM in literature (Table 1). Species highest in N include *Spirulina* spp. and *Chlorella* spp.. The N concentration instead of crude protein (CP) is reported in Table 1 to allow fair comparison between microalgae species and conventional feed resources. The non-protein N (NPN) concentration of microalgae (e.g. 2%–35% of

Table 1. Proximate composition (g/kg dry matter) of some microalgae species.

Phylum (common name), kingdom ^a	Species	Primary growth environment ^a	Ash	Nitrogen	Crude fat	NDF	α-linked glucose	Reference
Bacillariophyta (diatoms), kingdom: Chromista	<i>Phaeodactylum tricornutum</i>	Saline water	164 ^b (112–251) ^c	64 (29–83)	150 (74–223)	46 (28–64)	0.4 (0.3–0.5)	[9–14]
Bigyra, kingdom: Chromista	<i>Aurantiochytrium limacinum</i> , syn. <i>Schizochytrium limacinum</i>	Saline water	35 (28–40)	22 (19–26)	667 (608–743)	42	39	[15–20]
Chlorophyta (green algae), kingdom: Plantae	<i>Botryococcus braunii</i>	Fresh water	63 (54–72)	75 (57–89)	309 (110–471)	n.a.	n.a.	[21, 22]
	<i>Chlamydomonas reinhardtii</i>	Fresh water	109 (48–128)	54 (15–98)	141 (88–247)	n.a.	332 (73–693)	[12, 23–26]
	<i>Chlorella vulgaris</i>	Fresh water and terrestrial environments	112 (24–287)	68 (31–108)	155 (11–366)	55 (0–128)	115 (2.0–550)	[11, 13, 25, 27–36]
	<i>Chlorella pyrenoidosa</i>	Fresh water and terrestrial environments	80 (46–131)	89 (74–104)	158 (10–254)	75 (4–146)	n.a.	[33, 37–40]
	<i>Dunaliella salina</i>	Saline water	323 (90–720)	39 (12–64)	187 (101–281)	11 (0–21)	n.a.	[37, 41–43]
	<i>Haematococcus pluvialis</i>	Fresh water	54	53 (27–80)	277 (140–370)	n.a.	n.a.	[44–47]
	<i>Parachlorella kessleri</i> , syn. <i>Chlorella kessleri</i>	Fresh water and terrestrial environments	64 (56–71)	83 (75–91)	114	n.a.	n.a.	[12, 48]
	<i>Scenedesmus acutus</i>	Fresh water	80 (60–100)	88 (80–96)	130 (120–140)	n.a.	n.a.	[49]
	<i>Scenedesmus quadricauda</i>	Fresh water	48 (27–64)	82 (72–90)	138 (76–199)	n.a.	n.a.	[12, 43, 48]
	<i>Scenedesmus dimorphus</i>	Fresh water	88 (24–182)	83 (43–98)	120 (74–252)	n.a.	n.a.	[43, 50, 51]
	<i>Tetraselmis impellucida</i>	Saline water	173	56	231	n.a.	n.a.	[50]
	<i>Tetraselmis suecica</i>	Saline water	178 (146–207)	62 (27–85)	181 (80–307)	n.a.	n.a.	[12, 13, 52–56]
Cyanobacteria (blue-green algae), kingdom: Eubacteria	<i>Arthrospira platensis</i> , syn. <i>Spirulina platensis</i>	Fresh water	98 (66–195)	93 (26–114)	58 (8–116)	25 (0–120)	159 (35–609)	[11, 13, 27, 29, 37, 48, 57–68]
	<i>Arthrospira maxima</i> , syn. <i>Spirulina maxima</i>	Fresh water	96 (63–115)	99 (83–119)	61 (7–121)	n.a.	286 (52–700)	[49, 50, 63, 69–71]

Continued

Table 1. Continued.

Phylum (common name), kingdom ^a	Species	Primary growth environment ^a	Ash	Nitrogen	Crude fat	NDF	α-linked glucose	Reference
Euglenozoa, kingdom: Protozoa	<i>Euglena gracilis</i>	Fresh water	102 (35–140)	32 (18–46)	129 (81–193)	3.3 (0–6.5)	n.a.	[25, 72–75]
Haptophyta, kingdom: Chromista	<i>Isochrysis galbana</i>	Saline water	235 (160–310)	51 (32–73)	222 (162–274)	n.a.	0.1	[10, 76, 77]
	<i>Pavlova lutheri</i>	Saline water	n.a.	46	120	n.a.	n.a.	[76]
	<i>Pavlova salina</i>	Saline water	n.a.	42	120	n.a.	n.a.	[76]
Ochrophyta, kingdom: Chromista	<i>Nannochloropsis gaditana</i>	Saline water	104 (70–158)	53 (5–95)	118 (15–293)	99 (90–111)	14 (1–26)	[9, 11, 27, 50, 56, 78–81]
	<i>Nannochloropsis oculata</i>	Brackish water	85 (20–245)	56 (28–112)	283 (0–587)	n.a.	n.a.	[11, 25, 65, 82]
	<i>Nannochloropsis oceanica</i>	Saline water	192 (75–350)	55 (21–78)	236 (84–368)	288	0.3	[10, 13, 14, 83]
Rhodophyta (red algae), kingdom: Plantae	<i>Porphyridium cruentum</i>	Saline water and terrestrial environments	199 (168–236)	56 (34–90)	65 (58–76)	n.a.	n.a.	[11, 84, 85]
	<i>Porphyridium purpureum</i>	Saline and brackish water, terrestrial environments	204	53	88	n.a.	n.a.	[86]

n.a. = not available.

^a[87, 88].^bAverage value.^cMin – max. Only shown if more than one data point available.

total N; [28, 110]) has in some occasions exceeded those of rapeseed and soya bean meals (3%–17% of total N; [111–115]). Therefore, different N-to-CP conversion factors have sometimes been suggested for microalgae than for conventional feeds (e.g. 4.68–6.35 instead of 6.25; [84, 116, 117]). In contrast to monogastric animals, high dietary NPN concentration is not a problem for ruminants, although rumen fermentation and milk production seem to respond positively for the supply of true protein [118, 119].

Similarly to terrestrial plants, microalgae require C, N, P, S, K and many trace elements for their growth [120]. Nitrogen deprivation in the growth medium decreases the protein synthesis in microalgae [121], leading to decreased protein concentration in the biomass [122, 123]. Some, but not all cyanobacteria are able to fix atmospheric N, which makes the particular species less dependent on N supply from the environment or growth medium [124, 125]. Increasing NPN concentrations in *Chlorella vulgaris* have been reported with decreasing N concentrations in the growth medium, which might be related to the reduction of protein synthesis in microalgal cells [28]. Also, S supply can influence the protein concentration of microalgae [126]. In addition, the protein concentration of microalgae can vary depending on the growth phase; some species reach peak protein concentrations at exponential growth phase [127, 128], others at later phases of growth, that is, in early or late stationary phase [128].

Methionine, histidine and lysine are most interesting essential amino acids (EAA) in dairy cow nutrition, as their dietary supply is often limiting milk production [129–132]. Methionine and cysteine are important amino acids (AA) for wool production, and arginine and citrulline for gestation on ruminants [133]. However, any AA has the potential to limit milk, meat or wool production depending on the diet and its effects on microbial protein synthesis in the rumen [134]. In comparison to conventional feeds, microalgae are generally lower in histidine, but good sources of methionine (Table 2). It has sometimes been reported that cyanobacteria would be higher in valine, leucine and isoleucine and lower in lysine than diatoms and green algae, and lower in histidine and phenylalanine than green algae [149], but this was not observed here based on data reviewed in Table 2. Here we have only reviewed non-toxic *Arthrospira* spp., but the toxicity of cyanobacteria may also have an effect on the AA profile, as the toxins include AA like leucine and arginine [150].

It has been demonstrated with *Chlamydomonas reinhardtii*, a biological model species, that N starvation decreases the total concentration of amino acids [151], but the responses of individual amino acids depend on their physiological function in the algae. The changes in N supply cause no changes in the concentrations of most AA in *Chlamydomonas reinhardtii* [152]. However, the N-rich AA lysine, asparagine and especially arginine are very responsive to N supply; their concentrations decrease during N starvation, sparing N to other functions, and increase rapidly with increasing

N supply [152]. Especially free AA respond rapidly to external factors on cyanobacteria, green algae and diatoms [149]. Also on diatom *Navicula* sp. limited N supply has decreased the proportion of arginine whereas the proportion of other amino acids stayed relatively stable [153]. The concentrations of glutamic acid have been reported to increase during both N starvation and N supplementation [152]. Sulphur is taken by algae as sulphate, reduced to sulphide and immediately incorporated into cysteine, which is the precursor of all S-containing cellular compounds, including methionine [154]. Therefore, it is logical that short-term S-starvation has decreased cysteine, but not methionine concentration of *Chlorella sorokiniana* [126].

These physiological responses may explain some of the variation in amino acid composition within species in Table 2. There are also a considerable number of studies where microalgae AA profiles have been reported to be relatively stable in different phases of algae life cycle [155, 156] and under different growth conditions [28, 153, 157]. Different acid hydrolysis conditions during AA analysis can result in significant changes in the concentrations of microalgal AA, but it has not markedly changed the AA profile (proportion of individual AA of total AA) of a single species within an experiment [157]. Nevertheless, microalgal AA profiles deviating remarkably from those in Table 2 have sometimes been reported [84, 158, 159].

In addition to intracellular amino acids, some microalgae, like *Euglena gracilis*, are able to excrete amino acids into the culture medium at least in some cultivation conditions [160]. Excreted amounts of arginine, alanine, leucine, lysine and valine have been reported to exceed 30 mg/L in *Euglena gracilis*, and in terms of arginine, concentrations up to 80 mg/L have been observed. Microalgae also release other proteinaceous compounds to culture medium, like extracellular enzymes (e.g. carbonic anhydrases, proteases and phenoloxidases), protease inhibitors and phycoerythrin-like proteins [161]. Microalgae culture medium is currently a poorly utilised source of bioactive compounds [161].

For ruminant nutrition, the degradability of protein in the rumen is a very important characteristic that determines the availability of undegraded AA for intestinal absorption and affects the efficiency of conversion of feed N into milk protein. Generally, protein feeds with low ruminal protein degradability but high intestinal protein degradability are valued, which spares AA from microbial digestion in rumen but allows absorption from intestines for the host ruminant. *In vitro* ruminal protein degradability of different microalgae species is very variable [37, 57]. It has been reported to be 74% for non-cell-disrupted *Arthrospira platensis*, 79% for *Chlorella* spp., 54% *Nannochloropsis* spp. and 77% for *Phaeodactylum tricornutum* [57]. After 48-hour *in vitro* incubation, around 50% of the crude protein in *Chlorella vulgaris* was found rumen undegradable, and around 40%–48% of rumen undegradable protein was estimated to be intestinally digestible with some variation between microalgal cultivation conditions

Table 2. The amino acid composition (g/160 g N)^a of some conventional feeds and microalgae species.

Phylum	Species	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Cys	Reference
Bacillariophyta (diatom)	Soybean meal	73 ^b (60–91) ^c	27 (24–32)	46 (38–51)	77 (65–87)	62 (52–70)	14 (10–16)	51 (44–57)	38 (34–46)	14 (11–14)	48 (41–58)	16 (8–17)	[135]
	Rapeseed meal	58 (50–64)	27 (24–29)	40 (37–43)	68 (66–75)	53 (38–62)	20 (18–22)	39 (34–43)	43 (38–48)	12 (11–17)	51 (47–55)	24 (18–26)	[136]
	Fishmeal	58 (57–64)	22 (16–35)	43 (32–50)	70 (55–81)	75 (70–81)	28 (23–35)	38 (28–43)	41 (31–46)	11 (8–12)	49 (39–57)	8 (7–9)	[137]
	Wheat	47 (40–54)	23 (20–28)	34 (31–38)	65 (59–70)	29 (25–33)	16 (14–19)	45 (40–50)	29 (26–32)	12 (9–14)	43 (37–49)	22 (19–27)	[138]
	<i>Phaeodactylum tricornutum</i>	42 (29–50)	12 (9–17)	44 (35–63)	71 (54–100)	45 (41–49)	22 (20–28)	59 (37–111)	46 (37–62)	16 (13–20)	50 (39–67)	7 (4–11)	[10, 21, 57, 76]
	Bigyra	65 (32–78)	22 (20–22)	41 (23–46)	69 (39–78)	60 (39–66)	26 (12–34)	43 (27–48)	43 (22–51)	n.a.	62 (54–93)	34 (2–48)	[139, 140]
	Chlorophyta (green algae)	157	11	26	54	36	19	34	28	17	34	11	[21]
	<i>Botryococcus braunii</i>	102	34	74	172	91	44	103	86	0	103	21	[26]
	<i>Chlamydomonas reinhardtii</i>	57 (38–79)	20 (14–27)	35 (24–59)	78 (67–91)	54 (46–84)	19 (10–24)	53 (42–116)	38 (23–43)	15 (0.06–24)	49 (40–73)	9 (1–13)	[27–29, 35, 36, 141, 142]
	<i>Chlorella pyrenoidosa</i>	59 (47–69)	18 (14–20)	42 (33–53)	74 (50–87)	57 (47–68)	15 (5–34)	46 (41–54)	45 (39–49)	13 (10–18)	59 (54–67)	12 (8–17)	[142, 144]
Cyanobacteria	<i>Dunaliella salina</i>	n.a.	22 (21–24)	19 (1–48)	36 (5–80)	40 (30–64)	n.a.	n.a.	13 (4–32)	n.a.	18 (2–55)	n.a.	[145]
	<i>Dunaliella bardawil</i>	79	28	49	108	61	22	70	60	9	86	10	[146]
	<i>Haematococcus pluvialis</i>	44	13	23	55	29	n.a.	32	40	n.a.	32	n.a.	[46]
	<i>Parachlorella kessleri</i> , syn.	73	16	34	64	43	37	39	34	n.a.	42	33	[48]
	<i>Chlorella kessleri</i>	(62–83)	(15–16)	(34–35)	(63–64)	(43–44)	(37–37)	(39–39)	(32–37)		(42–43)	(32–33)	
	<i>Scenedesmus acutus</i>	58 (53–63)	16 (15–17)	39 (32–44)	82 (66–93)	54 (50–57)	15 (12–21)	43 (36–50)	52 (48–58)	13 (12–14)	65 (55–72)	10 (7–14)	[49]
	<i>Scenedesmus quadricauda</i>	43 (37–49)	16 (13–19)	24 (20–28)	53 (47–59)	52 (43–61)	40 (39–41)	37 (30–43)	29 (28–29)	n.a.	39 (31–47)	6 (26–27)	[48]
	<i>Scenedesmus dimorphus</i>	51 (50–54)	22 (20–22)	38 (37–42)	85 (81–91)	59 (58–60)	22 (21–24)	55 (52–59)	47 (43–52)	10 (7–21)	54 (52–58)	11 (10–12)	[50, 51]
	<i>Tetraselmis impellucida</i>	44	23	45	91	60	27	62	50	22	59	n.a.	[50]
	<i>Tetraselmis suecica</i>	60 (45–72)	18 (9–29)	39 (31–45)	82 (70–103)	48 (38–62)	24 (13–34)	75 (59–99)	46 (40–51)	10 (7–13)	48 (35–65)	10 (8–12)	[52, 55, 56, 76]
Euglenozoa	<i>Arthrospira platensis</i> , syn.	65	14	45	73	38	22	40	41	10	54	15	[27, 29, 48, 57, 58, 65, 144, 147]
	<i>Spirulina platensis</i>	(38–80)	(9–18)	(25–59)	(40–93)	(24–49)	(11–35)	(17–51)	(16–51)	(5–13)	(31–71)	(7–28)	
Euglenozoa	<i>Arthrospira maxima</i> , syn.	69 (61–78)	17 (15–19)	59 (56–60)	84 (73–93)	50 (46–52)	21 (13–28)	48 (43–50)	49 (42–55)	14 (12–15)	66 (62–76)	6 (4–8)	[49, 50, 71, 148]
	<i>Euglena gracilis</i>	64	26	39	80	66	20	44	60	17	63	15	[72]

Haptophyta	<i>Isochrysis galbana</i>	37 (32–41)	14 (11–17)	57 (51–62)	102 (92–112)	38 (31–45)	28 (25–31)	81 (57–106)	53 (46–60)	19 (14–25)	66 (61–70)	8 (6–10)	[10, 76]
	<i>Pavlova lutheri</i>	42	12	71	117	46	53	124	55	18	85	6	[76]
	<i>Pavlova salina</i>	37	8	57	116	45	22	101	60	9	69	6	[76]
Ochromytha	<i>Nannochloropsis gaditana</i>	59 (56–62)	19 (17–21)	44 (39–49)	82 (69–98)	61 (55–66)	19 (13–24)	49 (45–57)	46 (45–51)	19 (14–23)	53 (50–58)	8 (8–9)	[27, 50, 56]
	<i>Nannochloropsis oculata</i>	42 (33–52)	14 (11–17)	54 (47–62)	96 (91–101)	51 (45–57)	23 (21–24)	80 (55–104)	57 (50–64)	10 (3–17)	67 (60–74)	5	[65, 76]
	<i>Nannochloropsis oceanica</i>	54 (49–58)	16 (15–17)	52 (35–70)	79 (67–90)	71 (48–93)	18	46 (39–52)	46 (36–55)	17	97 (46–149)	7	[10, 83]
Rhodophyta (red algae)	<i>Porphyridium cruentum</i>	139	20	94	104	98	50	89	112	25	45	6	[84]
	<i>Porphyridium purpureum</i>	85	7	54	110	62	19	4	48	n.a.	76	3	[86]

n.a., not available.

^aIf no other information was provided, N-to-CP conversion factor of 6.25 was assumed for unit conversion.

^bAverage value.

^cMin–max. Only shown if more than one data point available.

[28]. *In vivo* data suggests that the ruminal protein degradability of *Arthrospira platensis* might be higher than that of rapeseed meal but lower than that of faba beans [29, 58]. In another *in vivo* study, *Arthrospira platensis* resulted in numerically higher ruminal ammonia concentration than isonitrogenously supplemented soya bean meal, which might indicate higher rumen protein degradability, but the difference did not reach statistical significance [162]. Effective degradability of N in ruminants has been reported to be 63%–71% for soya bean meal [135], 69%–77% for rapeseed meal [136] and 77%–80% for faba beans [163]. Mechanical cell disruption can increase *in vitro* ruminal protein digestibility, especially if the digestibility is low in the initial biomass [57].

Lipids

The lipid concentration (Table 1) and FA composition (Tables 3 and 4) of microalgae is highly variable between phylum and genera, and even within species, reflecting the plasticity and adaptability of microalgae to environmental conditions. Even the proportion of total FA from crude fat can vary greatly between and within species (Table 3); for example, in *Nannochloropsis gaditana* variation of 151–667 g/kg crude fat has been reported [9, 27]. Despite this vast diversity, 16:0 is usually one of the most abundant fatty acids across many microalgae genera (Tables 3 and 4). Typically, microalgae species within the same phyla and class have relatively similar FA composition [177]. High lipid concentration increases the energy density of the diet, which is favourable especially for the high yielding dairy cows. However, there is also limit to dietary lipid concentration in ruminant diets, as concentrations above 6%–7% in DM are associated with decreased DM intake and ruminal fibre digestibility, which may subsequently lead to decreased milk yield [226].

Microalgae contain many FA that are of interest in ruminant nutrition. Forages are typically the main source of omega-3 FA, especially α -linolenic acid (ALA; 18:3 n-3), for ruminants [174, 227, 228]. In contrast, omega-6 FA, mainly linoleic acid (LA; 18:2 n-6; 40%–60% of total FA), are major FA in concentrates like barley, oat, wheat [229], maize grain [230] and soybean meal [135]. Very-long-chain PUFA (>C18) cannot be synthesised in significant amounts in higher plants; in contrast, many algae and especially marine algae species are able to synthesise and accumulate high amounts of these FA, including arachidonic acid (20:4 n-6), eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) [231] (Table 4). Therefore, microalgae and fish oil derived from fish consuming microalgae are the most important sources of omega-3 very-long-chain PUFA for ruminants, although microalgal sources are still rarely used in practice. The use of fishmeal in ruminant nutrition is prohibited in EU (excluding milk replacers for young ruminants; EC No

999/2001) [232], Japan and Australia [233]. Especially, microalgae genera *Isochrysis*, *Nannochloropsis*, *Phaeodactylum*, *Pavlova* and *Thalassiosira* have been identified as potential substitutes for fish oil due to their high concentration of omega-3 long-chain PUFA, mainly EPA [234] (Table 4). Genera *Isochrysis*, *Pavlova* and especially species *Aurantiochytrium limacinum* are known to accumulate high concentrations of DHA (Table 4). Interestingly, some microalgae contain significant amounts of medium-chain SFA, mainly myristic acid (14:0), which are very rarely found in ruminant feeds (Table 4). Fatty acids 12:0 and 14:0 have been identified as very promising inhibitors of ruminal methane production that are reported to decrease ruminal methane emissions (g CH₄/kg energy corrected milk) up to 30% [103]. Conventional lipid supplements rich in 12:0 and 14:0 are typically very expensive making practical applications in commercial dairy farms unlikely [103], which may open an interesting market niche for microalgae production. Another FA rare in conventional ruminant feeds but rich in cyanobacteria (Table 4) is γ -linolenic acid (GLA; 18:3 n-6).

In many microalgae species, enhancing CO₂ supply and inducing stressful conditions, such as the limitation or even deprivation of nutrients (N, P, S), can generally cause the accumulation of N-free storage compounds such as lipids and starch or other polysaccharides into the biomass [94, 235, 236]. This is a well-known and largely utilised mechanism to boost microalgal lipid production. However, nutrient deprivation also decreases the growth rate and biomass production of microalgae, especially if long-term [236, 237]. The species, strain and environmental conditions determine whether the given microalga accumulates lipids, carbohydrates or both, and whether the accumulation of these storage compounds happens simultaneously or at different time scales [94, 236]. While N limitation is the most important method boosting microalgae lipid production, other environmental factors, like supply of minerals [238, 239], temperature [95, 240], light intensity [95, 241], production under photosynthetic or heterotrophic growth conditions [242, 243] and the growth phase of microalgae [244] can also affect the quantity and quality of microalgae lipids. The detailed description of complex effects of nutrient supply status and other environmental factors on microalgae composition are beyond the scope of this review, and the interested reader is referred to recent reviews [92, 94, 245] and references therein.

With sufficient N supply, microalgal lipid fractions are usually dominated by polar lipids (e.g. phospholipids, sphingolipids and glycolipids found mainly on membranes) [246, 247]. However, the proportion of neutral lipids (storage lipids such as triacylglycerols and sterol esters) typically increases with decreasing N concentration in the growth medium [231, 237, 246, 247]. The same response has been observed in other environmentally stressful growing conditions, too [235]. This contradicts the response on higher eukaryotes where abundant nutrient

Table 3. Total fatty acid content (TFA; g/kg crude fat) and saturated fatty acid composition (g/100 g total fatty acids) of some conventional feeds and microalgae species. Main fatty acids (>10 g/100 g total fatty acids) are bolded.

Phylum	Species	TFA	C4:0–8:0 ^a	10:0	12:0	14:0	16:0	18:0	20:0	22:0	24:0	Reference
	Soybean meal		0.05	0.1	0	0.1 ^b (0–0.2) ^c	15 (13–16)	4.2 (3.8–4.7)	0.2 (0–0.3)	0.4 (0.3–0.5)	0	[164, 166]
	Rapeseed meal		n.a.	n.a.	0.2	0.1 (0–0.2)	7.5 (6.1–9.2)	1.8 (1.4–2.3)	0.3 (0.3–0.4)	0	0.4	[165, 167–169]
	Wheat		n.a.	0	0	0.3	13	2.8	0.7	1.8	2.2	[170]
	Maize silage		n.a.	n.a.	0.2 (0.2–0.2)	0.4 (0.4–0.5)	16 (13–17)	1.4 (0–2.3)	0.8 (0–1.8)	0.5 (0.5–0.6)	0	[171, 173]
	C3 grass silage, pre-wilted, formic acid-treated		n.a.	n.a.	0.4 (0.2–0.6)	0.5 (0.4–0.7)	16 (15–17)	1.6 (1.2–2.0)	0.5 (0.5–0.6)	0.9 (0.9–0.9)	0.6 (0.6–0.7)	[27, 29, 174]
	Fish oil		n.a.	n.a.	0.1 (0.1–0.1)	6.6 (0.4–14)	15 (7.0–20)	3.0 (1.0–4.6)	0.4 (0.2–0.6)	0.2 (0.1–0.2)	0.2 (0–0.5)	[171–173, 175, 176]
Bacillariophyta	<i>Phaeodactylum tricornutum</i>	748 (713–783)	0.0	0.0	0.0	7.6 (3.0–16)	18 (8.5–26)	1.4 (0–5.5)	0.1 (0–0.4)	0.1 (0–0.5)	0.9 (0–2.6)	[9, 14, 57, 128, 177–180]
Bigyra	<i>Aurantiochytrium limacinum</i> , syn. <i>Schizochytrium limacinum</i>	810	0.0	0.0	0.1 (0–0.4)	3.2 (0.5–8.7)	38 (20–57)	1.2 (0.6–1.7)	0.3 (0.2–0.5)	0.2 (0.1–0.4)	2.1	[16, 20, 140, 181, 182]
Chlorophyta	<i>Botryococcus braunii</i>		1.3 (0.4–4.0)	n.a.	0.1 (0–0.7)	0.5 (0–1.3)	11 (6.4–17)	0.6 (0–1.3)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	[22, 177]
	<i>Chlamydomonas reinhardtii</i>		n.a.	n.a.	n.a.	0.3 (0–3.2)	25 (12–37)	2.2 (0–10)	0.0 (0–0.2)	0.0 (0–0.9)	0.0 (0–0.4)	[26, 177, 183–186]
	<i>Chlorella vulgaris</i>	269	3.2	n.a.	0.4 (0–0.9)	0.5 (0–2.0)	17 (7.7–25)	1.4 (0–6.5)	0.1 (0–0.3)	0.0 (0–0.1)	1.0 (0–4.0)	[27, 28, 33, 35, 36, 177, 187, 188]
	<i>Chlorella pyrenoidosa</i>		8.0 (2.8–12)	n.a.	1.2 (1.0–1.4)	0.8 (0.3–2.2)	19 (14–26)	1.2 (0.4–3.1)	0 (0–0)	n.a.	0 (0–0)	[33, 188]
	<i>Dunaliella salina</i>		0.1 (0–0.4)	0.0 (0–0.2)	0.1 (0–0.8)	0.5 (0–2.2)	13 (1.4–27)	0.5 (0–1.5)	0.2 (0–1.2)	0.3 (0–2.4)	0 (0–0)	[43, 177, 180, 189, 190]
	<i>Haematococcus pluvialis</i>		12 (2.9–21)	n.a.	0.6 (0.3–0.8)	0.7 (0–3.1)	18 (4.4–23)	3.2 (0–14)	0.4 (0–2.3)	0.1 (0–1.0)	0.3 (0–1.1)	[177, 191, 192]
	<i>Parachlorella kessleri</i> , syn. <i>Chlorella kessleri</i>		n.a.	0.0 (0–0.2)	n.a.	0.1 (0–0.8)	18 (3.0–32)	6.5 (0–72)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0.4)	[177, 193, 194]
	<i>Scenedesmus acutus</i>		n.a.	0.0	0.0 (0–0)	0.4 (0–1.7)	19 (13–30)	1.6 (0.4–2.3)	0.2 (0–0.7)	0.5	0.3 (0.2–0.4)	[195, 197]
	<i>Scenedesmus quadricauda</i>		n.a.	n.a.	0.0 (0–0.1)	1.5 (0–2.0)	9.7 (7.2–13)	0.5 (0.2–1.1)	0.1 (0–0.2)	0.1 (0–0.2)	0.1 (0–0.4)	[43, 195, 198, 199]
	<i>Scenedesmus dimorphus</i>		n.a.	n.a.	4.2 (0.1–5.3)	1.4 (1.0–1.8)	16 (12–30)	9.0 (1.2–13)	0.5 (0.4–0.7)	0.3 (0.3–0.4)	0.0	[43, 51, 200]
	<i>Tetraselmis impellucida</i>		n.a.	n.a.	n.a.	1.9	29	1.0	n.a.	n.a.	n.a.	[50]
	<i>Tetraselmis suecica</i>		n.a.	n.a.	n.a.	1.5 (0–8.0)	28 (13–50)	1.2 (0.2–3.3)	n.a.	n.a.	n.a.	[53, 128, 179, 189, 201]

Continued

Table 3. Continued.

Phylum	Species	TFA	C4:0–8:0 ^a	10:0	12:0	14:0	16:0	18:0	20:0	22:0	24:0	Reference
Cyanobacteria	<i>Arthrospira platensis</i> , syn. <i>Spirulina platensis</i>	598 (561–671)	2.3 (0–4.1)	3.9 (0–7.7)	0.4 (0–1.7)	0.3 (0–2.6)	40 (13–52)	0.9 (0–1.7)	0.0 (0–0.1)	0.0 (0–0)	0.1 (0–0.9)	[27, 57, 162, 177, 179, 188, 202, 203] (Lamminen et al., unpublished) [50, 177, 188]
	<i>Arthrospira maxima</i> , syn. <i>Spirulina maxima</i>		5.2	n.a.	0.5	0.2 (0–0.7)	30 (25–36)	1.9 (0–4.8)	0.0 (0–0)	0.0 (0–0)	0.2 (0–0.6)	
Euglenozoa	<i>Euglena gracilis</i>		n.a.	0.3	3.2 (0–7.2)	15 (0–79)	16 (4.1–33)	2.1 (0–7.4)	0 (0–0)	0.8 (0–14)	0.5 (0–6.1)	[72, 177, 204–206]
Haptophyta	<i>Isochrysis galbana</i>		n.a.	n.a.	0.0	16 (11–23)	25 (8.8–41)	2.6 (0–7.0)	0 (0–0)	0	0 (0–0)	[128, 177, 189, 207]
	<i>Pavlova lutheri</i>		n.a.	n.a.	n.a.	14 (10–35)	26 (15–41)	3.3 (0.7–11)	n.a.	n.a.	n.a.	[128, [208–[210]
	<i>Pavlova salina</i>		n.a.	n.a.	n.a.	15 (13–18)	16 (14–19)	0.4 (0.1–1.0)	n.a.	n.a.	n.a.	[180, 210]
Ochrophyta	<i>Nannochloropsis</i> <i>gaditana</i>	481 (151–667)	n.a.	0.1	0.3	5.0 (0–12)	28 (14–57)	3.9 (0–17)	0.1 (0–0.2)	0.3 (0–0.6)	0 (0–0)	[9, 11, 27, 50, 177, 189, 211, 212]
	<i>Nannochloropsis oculata</i>		n.a.	n.a.	n.a.	3.2 (0–8.4)	20 (8.5–33)	1.1 (0–2.3)	4.3 (0–6.8)	0	0	[177, 180, 189, 213–217]
	<i>Nannochloropsis oceanica</i>		n.a.	n.a.	1.2	4.5 (2.8–17)	27 (18–41)	9.9 (0.4–29)	0.2 (0–0.8)	0.4 (0.2–0.6)	0.0 (0–0.1)	[14, 178, 207, 218–220]
Rhodophyta	<i>Porphyridium cruentum</i>		n.a.	n.a.	0.0	0.7 (0–1.8)	35 (26–48)	0.7 (0.3–1.3)	n.a.	n.a.	0.0	[180, 207, 221–224]
	<i>Porphyridium purpureum</i>		n.a.	n.a.	n.a.	0.6 (0–5.2)	26 (1.6–37)	1.3 (0–3.4)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	[86, 177, 225]

n.a., not available.

^aShort-chain fatty acids, sum of 4:0, 6:0 and 8:0 fatty acids.^bAverage value.^cMin–max. Only shown if more than one data point available.

Table 4. Unsaturated fatty acid composition (g/100 g total fatty acids) of some conventional feeds and microalgae species. Main fatty acids (>10 g/100 g total fatty acids) are bolded.

Phylum	Species	16:1 n-7	16:4 n-3	18:1 n-9	18:2 n-6 (LA)	18:3 n-3 (ALA)	18:3 n-6 (GLA)	18:4 n-3	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)	Reference
	Soybean meal	0.1 ^a (0–0.1) ^b	n.a.	14 (14–15)	54 (52–55)	9.4 (8.6–10)	0	n.a.	0 (0–0)	n.a.	n.a.	[164–166]
	Rapeseed meal	1.0 (1.0–1.1)	n.a.	55 (48–63)	26 (23–30)	3.9 (2.7–6.0)	0	n.a.	0	n.a.	n.a.	[165, 167–169]
	Wheat	n.a.	n.a.	12	55	5.0	n.a.	n.a.	0.1	n.a.	n.a.	[170]
	Maize silage	0.3	0	17 (14–21)	43 (37–49)	7.7 (5.6–11)	0.3 (0–0.8)	0	0	0 (0–0.1)	0 (0–0)	[171–173]
	C3 grass silage, pre-wilted, formic acid-treated	0.2 (0.2–0.2)	n.a.	4.2 (3.3–5.1)	17 (16–19)	51 (48–55)	0	n.a.	0	0	n.a.	[27, 29, 174]
	Fish oil	4.3 (0.8–7.8)	0.2	8.7 (4.5–13)	1.5 (1.1–2.5)	1.3 (0.6–2.0)	0.7 (0.3–1.8)	3.0	1.4 (0.8–2.1)	19 (11–36)	19 (8.8–29)	[171–173, 175, 176]
Bacillariophyta	<i>Phaeodactylum tricornutum</i>	28 (14–42)	0.1	6.9 (0.5–19)	1.7 (0–3.9)	0.8 (0–3.4)	0.4 (0–0.7)	1.1 (0.5–3.6)	0.6 (0–2.4)	19 (6.3–33)	1.0 (0.2–1.9)	[9, 14, 57, 128, 177–180]
Bigyra	<i>Aurantiochytrium limacinum</i> , syn. <i>Schizochytrium limacinum</i>	0.1	n.a.	0.7 (0.1–1.3)	0.1 (0–0.4)	0.4 (0–0.6)	0.2 (0–0.3)	0.1	0.8 (0.1–1.5)	0.6 (0.3–1.3)	43 (27–65)	[16, 20, 140, 181, 182]
Chlorophyta	<i>Botryococcus braunii</i>	0.4 (0–1.1)	n.a.	39 (11–82)	3.8 (0–9.4)	9.8 (0–20)	0.0 (0–0)	0.0 (0–0)	0.2 (0–0.9)	0.8 (0–2.5)	5.3 (0–35)	[22, 177]
	<i>Chlamydomonas reinhardtii</i>	0.4 (0–2.7)	12 (0–26)	8.2 (0–26)	7.0 (0–18)	21 (0–42)	1.7 (0–7.1)	9.2 (2.1–29)	0.1 (0–0.9)	0.0 (0–0)	0.0 (0–0.5)	[26, 177, 183–186]
	<i>Chlorella vulgaris</i>	4.9 (0.4–18)	0.5	9.8 (1.5–45)	15 (0–49)	25 (2.3–43)	0.9 (0–4.5)	0.0 (0–0.1)	0.3 (0–1.1)	0.1 (0–0.9)	0.2 (0–2.8)	[27, 28, 33, 35, 36, 177, 187, 188]
	<i>Chlorella pyrenoidosa</i>	1.7 (0.9–3.7)	n.a.	13 (5.3–28)	13 (9.8–22)	27 (14–34)	0.2 (0–0.5)	n.a.	0.4 (0.1–1.0)	0.5 (0–1.3)	0.1 (0–0.2)	[33, 188]
	<i>Dunaliella salina</i>	0.5 (0–2.6)	26 (18–35)	3.2 (0–11)	8.9 (0–20)	35 (15–63)	1.4 (0–4.3)	0.5 (0–1.4)	1.4 (0–4.2)	0.1 (0–0.2)	0.8 (0–6.9)	[43, 177, 180, 189, 190]
	<i>Haematococcus pluvialis</i>	0.0 (0–0.4)	n.a.	12 (0–22)	27 (7.0–39)	14 (0.4–53)	1.8 (0–4.5)	2.0 (0–3.6)	1.7 (0–4.8)	1.0 (0–3.5)	0.6 (0–5.7)	[177, 191, 192]
	<i>Parachlorella kessleri</i> , syn. <i>Chlorella kessleri</i>	0.1 (0–0.9)	0.0 (0–0)	5.5 (0–19)	33 (0–62)	7.7 (0–21)	0.1 (0–1.4)	0.3 (0–5.9)	0.2 (0–4.4)	0.8 (0–3.6)	0.0 (0–0)	[177, 193, 194]
	<i>Scenedesmus acutus</i>	2.2 (0–3.3)	8.5 (5.1–15)	22 (6.8–39)	10 (7.2–12)	21 (13–35)	0.9 (0–1.5)	2.8 (2.2–3.3)	0.0 (0–0)	0.3 (0–0.8)	0.7 (0–2.1)	[195, 197]
	<i>Scenedesmus quadricauda</i>	1.1 (0–4.6)	25 (5.9–14)	9.1 (3.7–14)	9.8 (22–41)	32 (0.7–1.4)	0.9 (1.8–3.4)	2.6 (0–0)	0.0 (0–1.0)	0.3 (0–1.0)	0.0 (0–0)	[43, 195, 198, 199]
	<i>Scenedesmus dimorphus</i>	4.7 (3.5–5.9)	19 (12–18)	15 (4.3–18)	11 (19–29)	24 (0.2–2.1)	1.1 (0–0.2)	4.4 (0–0.2)	0.0 (0–0.3)	0.1 (0–0.3)	0.2 (0–0)	[43, 51, 200]
	<i>Tetraselmis impellucida</i>	4.8	n.a.	12	7.9	13	3.7	3.5	n.a.	3.2	n.a.	[50]
	<i>Tetraselmis suecica</i>	2.7 (1.0–5.0)	n.a.	11 (2.1–36)	4.8 (0–9.5)	12 (0–21)	0.7	7.1 (3.0–11)	0.8 (0–1.6)	3.7 (0–7.1)	0.0 (0–0)	[53, 128, 179, 189, 201]

Continued

Table 4. Continued.

Phylum	Species	16:1 n-7	16:4 n-3	18:1 n-9	18:2 n-6 (LA)	18:3 n-3 (ALA)	18:3 n-6 (GLA)	18:4 n-3	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)	Reference
Cyanobacteria	<i>Arthrospira platensis</i> , syn. <i>Spirulina platensis</i>	2.2 (0–6.5)	n.a.	5.0 (0–9.3)	15 (0–24)	1.4 (0–8.8)	19 (4.6–28)	0.0 (0–0)	0.0 (0–0)	0.2 (0–1.8)	0.9 (0–5.6)	[27, 57, 162, 177, 179, 188, 202, 203] (Lamminen <i>et al.</i> , unpublished) [50, 177, 188]
	<i>Arthrospira maxima</i> , syn. <i>Spirulina maxima</i>	5.1 (0.9–9.0)	n.a.	4.2 (1.9–6.1)	22 (16–33)	1.2 (0–4.9)	23 (18–25)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	0.0 (0–0)	
Euglenozoa	<i>Euglena gracilis</i>	0.9 (0–4.9)	n.a.	3.9 (0–8.1)	2.9 (0–16)	4.4 (0–19)	0.2 (0–2.9)	0.3 (0–4.0)	7.0 (0–19)	3.8 (0–14)	2.4 (0–13)	[72, 177, 204–206]
Haptophyta	<i>Isochrysis galbana</i>	15 (0–33)	n.a.	7.6 (2.5–17)	4.8 (1.6–9.6)	5.3 (0–19)	0 (9.8–16)	14 (0–0.2)	0.1 (0–0.2)	1.8 (0–6.9)	7.6 (0–20)	[128, 177, 189, 207]
	<i>Pavlova lutheri</i>	17 (11–33)	n.a.	4.5 (0–10)	1.3 (0–2.5)	0.7 (0–1.9)	0.8 (0.4–1.1)	6.4 (3.3–8.4)	0.2 (0–0.6)	15 (0–26)	8.8 (0–16)	[128, 208–210]
	<i>Pavlova salina</i>	11 (4.3–30)	0.0	1.1 (0.2–3.1)	1.8 (1.5–2.0)	1.0 (0–1.4)	1.6 (0.7–2.4)	11 (4.2–15)	1.4 (0.4–3.7)	25 (19–28)	8.4 (1.5–11)	[180, 210]
Ochrophyta	<i>Nannochloropsis gaditana</i>	21 (1.7–36)	n.a.	6.5 (0.3–25)	5.9 (0–20)	10 (0–33)	1.9 (0–3.6)	0.1 (0–0.1)	4.5 (1.7–8.8)	18 (0.2–38)	0.1 (0–0.3)	[9, 11, 27, 50, 177, 189, 211, 212]
	<i>Nannochloropsis oculata</i>	23 (6.8–32)	0.0	7.5 (0–37)	2.9 (0–10)	0.5 (0–2.0)	0.7 (0–1.9)	0.0 (0–0.1)	5.5 (0–11)	26 (13–36)	0.7 (0–3.2)	[177, 180, 189, 213–217]
	<i>Nannochloropsis oceanica</i>	18 (4.8–27)	0.0	16 (3.6–32)	3.4 (1.1–9.9)	5.0 (0–12)	n.a. (0–0.1)	0.0 (0–8.1)	4.3 (2.2–31)	16 (0–2.2)	0.8 (0–2.2)	[14, 178, 207, 218–220]
Rhodophyta	<i>Porphyridium cruentum</i>	1.1 (0.3–1.9)	0.0	0.7 (0.3–1.3)	6.3 (3.9–10)	0.2 (0–0.4)	0.4 (0.2–1.2)	0.0 (0–0)	24 (13–35)	24 (16–41)	0.0 (0–0.1)	[180, 207, 221–224]
	<i>Porphyridium purpureum</i>	1.0 (0–4.1)	n.a.	5.0 (0–18)	11 (0.3–24)	8.4 (0–23)	0.0 (0–0)	0.0 (0–0)	19 (0.5–45)	15 (10–27)	0.0 (0–0)	[86, 177, 225]

n.a., not available.

^aAverage value.^bMin–max. Only shown if more than one data point available.

supply increases the concentration of neutral lipids (i.e. storage lipids) [248].

The FA profile of polar and neutral lipids is not identical in microalgae [59, 247, 249]. For example, *Arthrospira platensis* lipids are found mainly in polar fraction as phospholipids and glycolipids, but the major part of some individual FA, like 16:0 and 18:0, is found in neutral lipid fraction [59]. As the solubility of different lipid fractions into various solvents also varies [250], relatively different lipid concentrations and FA profiles for the same microalgae batch might be obtained depending on the solvent used in lipid extraction [59, 218, 251–253]. Even the applicability of selected gas chromatography method can influence the microalgal FA profile [254].

The abovementioned examples highlight the importance of basic understanding of microalgal biology also in animal nutrition, and call for transparency in reporting the origin of microalgae and the analytical methods used in lipid analysis in order to increase the replicability of research. However, they have also direct impacts on animal nutrition, because both microalgae lipid supplements and lipid-extracted biomass can be utilised as feed resources. Solvents can also be used to remove odoriferous compounds in microalgae in order to increase their acceptability for humans and animals [59]. Solvents like hexane, methanol and chloroform are most often used in lipid extraction, but new methods are also being developed, for example, the use of ethanol and cyclopentyl methyl ether as solvents [255]. Ethanol and acetate extraction has triggered only minor changes in the FA profile of *Arthrospira platensis* biomass; however, hexane extraction resulted in complete removal of 16:0 and 18:0 and increased by nearly two-fold the proportion of LA and GLA in total FA [59]. The EPA concentration of *Nannochloropsis* spp. oil has varied up to 15%–20% units depending on the solvent used in lipid extraction [252, 253].

The purpose of microalgae production largely determines the aims of tailoring the FA composition. The high concentration of PUFA is desirable in animal production in order to create potential health effects in animals and humans consuming the animal-derived products. On the other hand, the opposite is true for biodiesel production as the increase of FA unsaturation rate decreases the oxidative stability of the product [256]. For example, the current standards of European Union for biodiesels (EN 14214 and EN 14213) require that the content of FA methyl esters with ≥ 4 double bonds is less than 1% (mol/mol) and the content of C18:3 (ALA and GLA) in biodiesel intended for vehicle use is lower than 12% (mol/mol) [256, 257].

Another interesting aspect related to the use of microalgae as lipid supplements for ruminants is related to their cell wall and microscopic size. Nutrients in intact microalgae are often protected by cell wall, which can also hinder or prevent the biohydrogenation of microalgal lipids; therefore microalgal biomass and extracted microalgal oil can behave differently in the rumen [258]. It

has also been reported that the biohydrogenation of EPA and DHA in microalgae *Schizochytrium* sp. is lower than fish oil [227]. The structure of the microalgal cell wall varies greatly between species [6]. Therefore, it is logical that also the susceptibility of microalgal FA for ruminal biohydrogenation differs between species [178, 259]. Not much is currently known about the ruminal passage kinetics and the biohydrogenation processes of unicellular microalgae; therefore the possible mechanisms can only be speculated. The ruminal passage rate of microalgae might also resemble more that of liquid than regular feed particles due to very small microalgal cell size [179] and easy flowability in liquid. The latter is caused by microalgal cell density close or similar to that of water [260]. In that case, it is possible that the retention time of microalgae in the rumen might be shorter than that for other feed components due to faster passage rate of liquid phase [261]. Subsequently, the digestion process of microalgae might shift to lower parts of gastro-intestinal tract, which also protects FA from ruminal biohydrogenation. Microalgal FA might also be able to escape ruminal biohydrogenation because the efficiency of lipolysis of triglycerides and hydrogenation of FA decreases with decreasing particle size due to declining adherence of bacteria to feed particle surface [262]. Environmental conditions (e.g. N supply) also affect the cell size and cell wall thickness of microalgae [263], which can have direct effects on digestibility of nutrients and ruminal biohydrogenation of lipids in microalgae.

Carbohydrates and fibrous components

Storage carbohydrates

The primary storage carbohydrates found in feeds of non-animal origin are constituted of glucose or fructose subunits with varying bond type that links molecules together. Three main types of storage polysaccharides are found in conventional ruminant feeds: starch (α -(1–4) and α -(1–6) linked glucose) in the majority of higher plants [264] including sub-tropical and tropical grasses [265, 266], fructans (β -linked fructose) in temperate grasses [265, 266] and sucrose (glucose and fructose joined by α -1, β -2 glycosidic linkage) in grasses [265] and some by-products of sugar production [267]. In contrast, there is huge variation in the form of storage carbohydrates between different microalgae genera and it may even vary from species to species within the genera. Sometimes the storage polysaccharides of microalgae are simply stated to contain either α (glycogen or starch) or β -linked (chrysolaminarins and paramylon) glucose molecules (glucan), of which glycogen and chrysolaminarins are water-soluble and starch and paramylon solid crystalline forms [268]. However, as we see in the following section, and which was also demonstrated in [268], microalgal storage polysaccharides are much more complex. The

structural features of storage polysaccharides (e.g. amylose:amylopectin ratio, granule size and gelatinization temperature in starch) affect the physical quality and digestibility of processed feeds [269]; therefore they are important characteristics for the feed industry. Here the storage carbohydrates of microalgae are briefly covered in the sequence of evolutionary history of the phyla, as the complexity of compounds increases with endosymbiotic events. Focus is on the phyla described on Tables 1–4. For more information about the environmental impacts on storage carbohydrate composition, the reader is referred to previously published review [270]. A broad term ‘ α -linked glucose’ is used in Table 1 instead of starch. The starch analysis is typically based on the release of α -(1–4) and α -(1–6) linked glucose from a starch molecule by α -amylases and amyloglucosidases [271]. However, other polysaccharides with similar structure or identical physical behaviour to starch may also be hydrolysed in this process [271]. These include glycogen, maltodextrins, maltose, isomaltose, α -glucan [271] and floridean starch [272]. This challenge is not unique to microalgae, but the comparison of microalgae species is complicated by the diversity of their storage carbohydrates.

In most cyanobacteria, polysaccharides are stored as water-soluble glycogen (α -(1–4) linked glucose) [91, 273–275]. However, a few nitrogen fixing cyanobacteria species store polysaccharides as an insoluble semi-amylopectin, which is an intermediate-type polyglycan between glycogen and amylopectin [274, 275]. Some of the species synthesising semi-amylopectin also contain amylose, forming a starch-like substance [275]. Cyanobacteria are also able to synthesise sucrose, trehalose, glucosylglycerol, glucosylglycerate and glycine betaine, but their production seems to function mainly as a salt acclimation mechanism rather than storage polysaccharide [276]. Sucrose also functions as a carbon-carrier molecule in N-fixing cyanobacteria [276]. The glycogen concentration of *Arthrospira platensis* has varied 35–609 g/kg DM (on average 159 g/kg DM) depending on production conditions (Table 1), and it represents a major part of total carbohydrates found on this species [60, 277]. Especially N depletion increases the glycogen concentration of *Arthrospira* spp. [69, 278].

Green algae include phyla Chlorophyta and Streptophyta, of which land plants have evolved [279]. Due to their shared evolutionary history, Chlorophyta store carbohydrates as starch, similarly to terrestrial plants [91, 275]. In addition, the composition of starch is similar to terrestrial plants constituting of amylopectin and amylose [273]. Even the genes and their functions involved in starch metabolism have remained unchanged during the evolution from ancestral unicellular green algae to complex terrestrial plants [273, 280]. However, there might be some variation in storage carbohydrate composition of Chlorophyta, as it has been observed that *Scenedesmus obliquus* contained semi-amylopectin instead of amylopectin [281]. Starch concentrations up to 603 g/kg DM have been reported in *Chlorella* sp. [282]. Starch in *Chlorella sorokiniana*

was characterised by small granule size (around 1 μ m) with narrow size distribution and similar crystallinity pattern (A-type) to cereal starch [283]. Amylose contents of 170 g/kg and 340–380 g/kg have been reported for starch in *Chlorella sorokiniana* [283] and *Chlorella* sp. [284], respectively. This is close to regular wheat and corn, in which amylose content of starch typically ranges 180–300 and 220–330 g/kg, respectively [285]. The gelatinization of *Chlorella sorokiniana* starch has started at 24°C, peaked at 69°C and completed at 97°C [283]. The onset temperature of gelatinization in *Chlorella sorokiniana* starch is much lower, peak temperature similar and final temperature higher than on potato, corn, rice and wheat starch [285]. Resistant starch is the proportion of starch that cannot be degraded by amylolytic hydrolysis, and thus is not digested in small intestines. No resistant starch has been found in *Chlorella* sp. [286], which is beneficial for monogastric nutrition. However, in ruminants, slow degradation rate of starch and starch resisting microbial enzymatic degradation in the forestomachs is beneficial for rumen function as it lowers the risk of acidosis [287].

Rhodophyta (red algae) includes two subclasses, Florideophycidae and Bangiophycidae, which differ slightly in the produced storage carbohydrates [281, 288]. As unicellular (microalgae) species are only found in subclass Bangiophycidae [281], this review focuses on their carbohydrate composition. Traditionally, floridean starch (α -polyglucan consisting only of amylopectin and no amylose) has been considered the main storage carbohydrate in all Rhodophyta, but new research especially with unicellular Bangiophycidae has diversified this conclusion [281, 288, 289]. Storage carbohydrates in unicellular Rhodophyta are diverse, as they contain species that produce glycogen or alternatively amylose and semi-amylopectin as their storage carbohydrates [281, 288, 289]. It has even been suggested that the type of accumulated storage carbohydrate in Rhodophyta may vary depending on the growing conditions [288].

Bacillariophyta, Haptophyta and Ochrophyta store carbohydrates as a chrysolaminarin [91, 268, 290]. Chrysolaminarin is predominantly β -(1,3) linked glucan with occasional β -(1,2), β -(1,4) or β -(1,6) branching, and is easily extractable by dilute acid or hot water [291]. Chrysolaminarin concentrations of 40–300 g/kg DM have been reported during exponential growth phase and up to 810 g/kg DM during the stationary phase [292]. Nitrogen limitation and high light intensities also promote the accumulation of chrysolaminarin (up to 500 g/kg DM) in diatoms [291]. Ochrophyta have no ability to metabolise α -glucans [268]. Some studies have reported small amounts of α -linked glucose in *Bigyra* (Table 1). However, the storage polysaccharide metabolism of this phylum is largely unknown, as according to phylogenetic analysis of enzymes involved *Bigyra* have lost the ability to both α - and β -glucan metabolism [268].

Euglenophytes store carbohydrates as an insoluble, highly crystalline fibrillary β -(1,3) linked glucan called

paramylon [91]. The crystallinity of paramylon is comparable to that of cellulose [291] and it is classified as a dietary fibre [293]. Paramylon concentrations of 15–532 g/kg DM have been reported with *Euglena gralicis* [294, 295].

Cell walls and structural carbohydrates

Not much is known about the fibrous fractions of microalgae described as detergent fibre (neutral detergent fibre NDF, acid detergent fibre ADF, acid detergent lignin ADL) (Table 1), which are typically used to evaluate the availability of feed nutrients for livestock. This may relate to the challenges with the analytical methods involving the use of filters when analysing microalgae (crucibles or nylon bags in detergent fibre analysis, *in vitro* digestion method and *in situ* rumen incubation). As unicellular organisms, the microscopic particle size of microalgae is much smaller than the pore size of crucibles (recommendation of 40–90 µm, [296]) or nylon bags (often 17–37 µm, [297, 298]) used in these analytical methods; thus there is a significant risk of washout. Nylon bags with pore size less than 10 µm are not even recommended for incubations involving ruminal liquid, as it can restrict the number of rumen protozoa and bacteria entering the digestion bags [298]. The cell diameter of various spherical microalgae species has been reported to be 2.5–5.6 µm, and while the length of microalgae with spindle, oval or filament cell shape has varied 9–500 µm, their width has been reported to be only 3–10 µm [179]. The change of crucible pore size from 40–100 µm to 16–40 µm resulted in higher NDF concentrations of *Arthrospira platensis* (0 vs. 87 g/kg DM), *Chlorella vulgaris* (0 vs. 15 g/kg DM) and *Nannochloropsis gaditana* (90 vs. 219 g/kg DM) [27]. This highlights the importance of filter pore size in microalgae analysis and warrants further development of the standard analytical methods used in feed evaluation to suit better for the analysis of microalgae. However, regardless of the NDF concentration in microalgae, microalgal NDF likely has no relevance for maintaining rumen function (i.e. microalgae has no physically effective fibre) given the tiny particle size. Even though most of the scarce data on detergent fibre composition of microalgae indicates very low concentrations, very high concentrations are sometimes reported for lipid-extracted microalgae products (e.g. 470 and 47 g/kg DM of NDF and ADF, respectively, for lipid-extracted *Chlorella sorokiniana* residue; [299]).

The unfamiliar algal polysaccharides that are not found in conventional feeds may complicate detergent fibre analysis also when small particle size is not an issue. For example, the ADF fraction (i.e. lignin, cellulose) of brown seaweeds has been reported to be higher than NDF fraction (i.e. hemicellulose, cellulose, lignin) [300], which should be impossible by the definition of detergent fibre method [301]. It is hypothesised that alginate, which is found in high concentrations in brown seaweeds, is accumulated to ADF fraction instead of NDF [300]. Brown

algae do not contain any microalgae species; hence, no alginate containing microalgae exists according to current knowledge. Nevertheless, the possibility that other polysaccharides in microalgae may cause challenges with detergent fibre method cannot be ruled out. Situations with ADF higher or very close to NDF have also been reported with microalgae, although the concentrations have been so small that the difference might also be caused by analytical imprecision [37]. Of compounds unique for algae, the storage polysaccharide paramylon (classified as a dietary fibre) seems not to accumulate on detergent fibre fraction, since very low concentrations of NDF (0–6.5 g/kg DM) and ADF (0–2.8 g/kg DM) have been reported with *Euglena gracilis*, species rich in paramylon [72, 73].

Due to the lack of information on detergent fibre composition of microalgae, the chemical composition of microalgal cell walls is reviewed. This knowledge is helpful when narrowing down the species having most potential at animal nutrition applications. In ruminant diets, the digestibility of NDF varies depending on its monosaccharide composition: NDF glucose > NDF xylose and uronic acid > NDF arabinose, galactose and mannose [302]. The understanding of ruminal digestibility of microalgal cell walls is also important from industrial perspective, as microalgae biomass can be pre-treated with ruminal microorganisms in order to increase the yield of gaseous biofuel production [303].

While the cell walls of terrestrial plants constitute mainly of carbohydrates (especially cellulose), phenolic compounds and only minor amounts of protein (up to 10%) [304], the cell wall structure and composition of microalgae can be very variable, as visualised by [6]. However, the cell wall composition of only a limited number of microalgae species is studied and their influence on the accessibility of microalgal nutrients and other valuable compounds is not yet fully understood [89, 305]. The cell wall polysaccharides of microalgae typically form around 100 g/kg of cell DM, although variation is large (from 35 g/kg DM in *Thisochrysis lutea* to 170 g/kg DM in *Tetraselmis chuii*) [30]. So far, the presence of true lignin in green and red macroalgae (seaweeds) could not have been confirmed [306]. Therefore, true lignin will unlikely be found in unicellular microalgae either, which are simpler in structure than multicellular algae. Nevertheless, the enzymes involved in making the simplest lignin monomers have an ancient origin and genes associated in their biosynthesis are found in a variety of eukaryotic microalgae phyla [307]. Indeed, some lignin-like phenolic compounds have been reported on macroalgae, simple multicellular algae [306, 308] and even on some microalgae [309].

The cell walls of cyanobacteria are constituted of peptidoglycan (also called as murein) [310, 311], which is a polymer composed of *N*-acetylglucosamine, *N*-acetylmuramic acid and several AA [311]. Polysaccharides are likely found only as minor components in cell walls of *Arthrospira platensis* [30]. Cyanobacterial cell walls have structural features both of gram-negative and gram-positive bacteria

[310], but they differ in cell wall thickness. Thicknesses of 1.5–10 nm and 20–80 nm have been reported for gram-negative and gram-positive bacteria, respectively [312]. In contrast, the thickness of cyanobacterial cell walls can range from 10 nm to more than 700 nm [310]. Some cyanobacteria also have cellulose-containing minor extracellular components as slime tubes, sheaths and extracellular slime [313].

The diversity of cell wall structures and composition in Chlorophyta is tremendous, not only varying between species and strains, but also in different life stages of the cell [6, 89, 305]. Many different grouping systems have been suggested for Chlorophyta based on the chemical composition of their cell walls. *Chlorella* spp. have been divided into species having glucosamine-rigid cell wall (e.g. *Chlorella vulgaris*) and species with glucose-mannose rigid wall (e.g. *Chlorella pyrenoidosa*) [314, 315]. Chlorophyta also contain species that completely lack a cell wall and have only plasma membrane, like *Dunaliella salina* [6, 89, 316]. In *Chlorella vulgaris*, the rigid cell wall fraction has constituted of 60%–66% of the cell wall DM, and hemicellulose 23%–25% [317]. However, the proportions change during the growth phase, as the mass of rigid fraction stays constant but the mass of hemicellulose increases with cell age [89]. In other type of classification, Chlorophyta have been divided into three groups: (1) species with cell walls constituting of 2-keto-sugar acids (Parasinophytina and Chlorodenrophyceae); (2) species with cell walls constituting of mannans, glucans, chitin-like polysaccharides and algaenan layer (unicellular Trebouxiophyceae and Chlorophyceae) and (3) species with cell walls constituting of xylan, mannan, glucan and sulphated and/or puruvylated polysaccharides (macroalgae species, i.e. seaweed) [89]. Algaenans may pose a challenge for digestion, as they are highly resistant to alkali and acid hydrolysis and aqueous and organic solubilisation [318]. Algaenan has been found on Chlorophyta genera *Scenedesmus*, *Tetraedron*, *Chlorella*, *Botryococcus* and *Haematococcus* as reviewed by [319]. The fibrous material in Chlorophyta can be based on cellulose or chitin-like polysaccharide (β -(1 \rightarrow 3)-xylan or β -(1 \rightarrow 4)-mannan) [89]. *Chlorella* spp. can also contain pectin [320]. Lipid-extracted *Chlorella sorokiniana* residue has contained 420 g/kg DM of hemicellulose, 27 g/kg DM of cellulose and 26 g/kg DM of lignin [299]. The protein concentration of cell walls in Chlorophyta varies largely: from 2% in *Chlorella vulgaris* [317] to 32% in *Neochloris oleoabundans* [321]. Due to their vast diversity, thus far no single industrial process has been found that is applicable to treat with success the cell walls of all Chlorophyta [89]. This is also reflected to *in vitro* rumen DM digestibility of Chlorophyta, which has varied considerably between species (654–797 g/kg DM; [259]). In another study examining the same Chlorophyta species, great differences were found in two-phase gastric/pancreatic *in vitro* digestibility (494–778 g/kg for CP) designed for monogastric applications; in contrast, no or only minor differences were found *in vitro* rumen organic matter digestibility [322].

The cell wall characteristics of unicellular Rhodophyta are less known than the multicellular macroalgae species of the phylum [323]. The cell wall of *Porphyridium purpureum* consists of two layers: the inner cellulose and the outer mucopolysaccharide layer with varying thickness depending on the growth stage [324]. The cell wall of *Porphyridium* spp. and *Dixonella grisea* contains sulphated polysaccharides, whose complete structure is still unknown due to their complex structures and lack of specific degrading enzymes [323, 325]. The main sugars in these polysaccharides are xylose, glucose and galactose with varying ratios [323, 325]. Glycoproteins with novel structures are bound to the cell wall polysaccharides on microalgal Rhodophyta [323].

Very little information is available on the cell wall characteristics of Haptophyta. Many Haptophyta are covered by scales consisting of organic material having cellulose as the main component [291]. Some species with coccoid growth stages have calcified scales (coccoliths) [291] that consist of calcium carbonate [326]. The contribution of cell wall polysaccharides to cell DM is low in *Tisochrysis lutea* [30]. It has also been reported that *Isochrysis galbana* would have only plasma membrane covering and no cell wall [316].

The ornamental cell walls of Bacillariophyta (diatoms) come with many shapes and patterns [327]. The cell walls (frustule) of diatoms have three layers: (1) the innermost organic layer, (2) silicified mineral layer containing also organic matter and (3) external organic coat that is covered with mucilage [290]. The organic matter in diatom cell wall is mainly composed of polysaccharides, proteins and polyamines [290]. The polysaccharides of *Phaeodactylum tricornutum* consist of mannose, glucuronic acid residues and sulphate groups [328]. Genera *Cyclotella* and *Thalassiosira* contain also highly crystalline fibres of β -chitin [290]. The rigidity of the diatom cell wall can pose challenges for its own growth and cell division [327], and cell disruption may be beneficial when fed to ruminants as it has been reported to slightly increase organic matter digestibility, intestinal digestibility of rumen undegradable CP and *in vitro* total volatile fatty acid and gas production [28].

The cell walls of Ochrophyta species *Nannochloropsis gaditana* have bilayer structure with cellulosic inner wall (around 750 g/kg of cell wall DM), which is covered by algaenan layer [318]. The algaenan outer wall causes the rigidity of the cell walls of *Nannochloropsis* spp., as it is chemically and enzymatically very resistant material (see above). Around 60 g/kg of cell wall DM of *Nannochloropsis gaditana* is consisted of amino acids [318]. Ochrophyta contain also species with silica scales and species with an envelope called lorica, which is mainly composed of chitin and cellulose [329].

The cell walls of Bigyra species *Schizochytrium aggregatum* and *Thraustochytrium* sp. are reported to contain 210–360 g/kg DM of carbohydrate and 300–430 g/kg DM protein [330]. Cell wall monosaccharides in *Schizochytrium aggregatum* are primarily constituted of L-galactose

(>950 g/kg) and in *Thraustochytrium* of galactose (around 660 g/kg) and xylose (around 330 g/kg), the L-galactose being the predominant form [330].

Euglenophyta have no cell wall, but unusual cell membrane complex, pellicle, which is consisted of proteinaceous strips, microtubules and tubular cisternae of endoplasmic reticulum being located underneath the plasma membrane [329]. The pellicle mainly consists of protein (around 680 g/kg DM, [331]). *Euglena gracilis* has a complex glycan surface consisting of galactan, xylan and aminosugars, but the protein glycosylation is simple with high mannose-type glycans [332].

Conclusion and future research

The tremendous diversity of microalgae between and within species enables multiple utilisation and tailoring opportunities for sustainable ruminant production. Species with high protein concentration have potential to substitute conventional protein feeds in ruminant diets. The knowledge on ruminal degradability of microalgal protein and the influence of different microalgae production conditions on it is still scarce, although ruminal degradability of protein is one of the key characteristics of a feed determining the protein use efficiency of the ruminant. Microalgae are generally rather high in methionine, but lower in histidine than conventional protein feeds. However, it seems possible, at least to some extent, to tailor the AA composition (arginine, asparagine, cysteine, glutamic acid and lysine) of microalgae with N supply on the growth medium. On the other hand, N supply can also have dramatic influence on the lipid:protein or carbohydrate:protein ratio, NPN concentration and lipid profile of microalgae. Therefore, changes of growth medium nutrient supply to the complete composition of microalgae must be carefully evaluated before any specific recommendations can be given for tailoring microalgae production to specific animal production targets. Microalgae with high concentrations of very-long-chain PUFA (e.g. EPA, DHA) can potentially improve the FA profile of milk and meat for human consumption. The possibility to decrease enteric methane emissions with microalgae having high concentrations of 12:0 and 14:0 FA is currently completely underexploited area of research. The production objectives of microalgae for biodiesel and animal production and human consumption may sometimes be in conflict, as the aim is to limit PUFA concentration for biofuel production, but often to maximise it for animal and human nutrition. Very little is known about the NDF and ADF composition of microalgae and the development of the standard feed evaluation methods involving the use of filters (NDF, ADF, ADL, *in vitro* digestion, *in vivo* incubation) is urgently needed in order to suit better for the analysis of unicellular microalgae with microscopic particle size. More knowledge is needed also about the ruminal passage kinetics and the biohydrogenation processes of microalgae,

as they directly influence the transfer efficiency of FA from feed to milk and meat and N metabolism of the ruminant. In order to realise the full potential of microalgae in ruminant diets, the economic competitiveness of microalgae needs to be improved with further technical advantages of microalgae production, but also the development of microalgae feeding technique for animals (e.g. via drinking water or as a wet paste) might provide some cost savings for microalgae production. In the future, the possibility to produce added-value products or mitigate climate change via microalgae feeding of ruminants might also provide interesting opportunities for improving economic competitiveness of microalgae.

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